

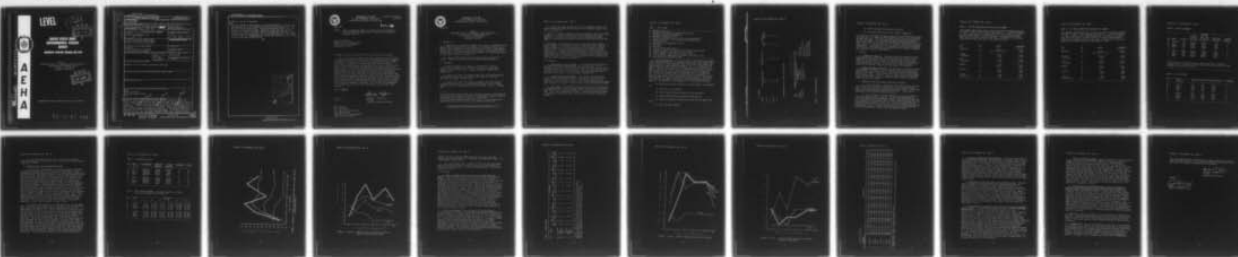
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ARMY ENVIRONMENTAL HYGIENE AGENCY ABERDEEN PROVING GR--ETC F/G 6/20  
BEHAVIORAL EFFECTS OF SUBCHRONIC AEROSOL EXPOSURE TO N,N-DIETHY--ETC(U)

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USAEHA-75-51-0034-80

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PHASE 3  
BEHAVIORAL EFFECTS OF SUBCHRONIC AEROSOL EXPOSURE TO  
N,N-DIETHYL-META-TOLUAMIDE (M-DET)  
STUDY NO. 75-51-0034-80  
FEBRUARY - MAY 1979

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| 20. ABSTRACT (Continue on reverse side if necessary and identify by block number)<br>Groups of 10 male and 10 female rats were exposed to aerosols of M-Det (an insect repellent) for 6 hours per day, 5 days per week, for 13 weeks at concentrations of 1,500 mg/m <sup>3</sup> (1/4 LC50), 750 mg/m <sup>3</sup> (1/8 LC50), 250 mg/m <sup>3</sup> (1/24 LC 50), or chamber air. The rats were given balance, tactile sensitivity and endurance tests during weeks 2, 4, 6, 8, 11, and 13 of the study. During the 13th week, they were also given memory, learning and activity tests. The results of the battery permitted distinctions to be made among performances at all three levels and controls for both males and females. Results of most tests repeated throughout |                       |  |

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Item 20. Abstract (continued).

the study changed in various ways as the study progressed. No toxic signs were observed in the participants when behavioral tests were carried out, and none died during the study. No gross abnormalities were evident upon necropsy at the end of the study, and groups of the same sex remained statistically similar in weight. Results of hematologic, histopathologic, chemical, and physiological tests will be reported separately. No changes in use of M-Det are recommended until further tests are run by other laboratories testing lower concentrations.

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DEPARTMENT OF THE ARMY  
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY  
ABERDEEN PROVING GROUND, MARYLAND 21010

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HSE-LT/WP

SUBJECT: Phase 3, Behavioral Effects of Subchronic Aerosol Exposure to  
N,N-Diethyl-Meta-Toluidine (M-Det), Study No. 75-51-0034-80,  
February - May 1979

Executive Secretary  
Armed Forces Pest Control Board  
Forest Glen Section, WRAMC  
Washington, DC 20012

A summary of results and conclusions of the inclosed report follows:

Groups of 10 male and 10 female rats were exposed to aerosols of M-Det (an insect repellent) for 6 hours per day, 5 days per week, for 13 weeks at concentrations of 1,500 mg/m<sup>3</sup> (1/4 LC50), 750 mg/m<sup>3</sup> (1/8 LC50), 250 mg/m<sup>3</sup> (1/24 LC50), or chamber air. The rats were given balance, tactile sensitivity and endurance tests during weeks 2, 4, 6, 8, 11, and 13 of the study. During the 13th week, they were also given memory, learning and activity tests. The results of the battery permitted distinctions to be made among performances at all three levels and controls for both males and females. Results of most tests repeated throughout the study changed in various ways as the study progressed. No toxic signs were observed in the participants when behavioral tests were carried out, and none died during the study. No gross abnormalities were evident upon necropsy at the end of the study, and groups of the same sex remained statistically similar in weight. Results of hematologic, histopathologic, chemical, and physiological tests will be reported separately. No changes in use of M-Det are recommended until further tests are run by other laboratories testing lower concentrations.

FOR THE COMMANDER:

BRENDAN E. JOYCE, Ph.D.  
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DEPARTMENT OF THE ARMY  
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ABERDEEN PROVING GROUND, MARYLAND 21010

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PHASE 3  
BEHAVIORAL EFFECTS OF SUBCHRONIC AEROSOL EXPOSURE TO  
N,N-DIETHYL-META-TOLUAMIDE (M-DET)\*†  
STUDY NO. 75-51-0034-80  
FEBRUARY - MAY 1979

1. AUTHORITY.

a. Memorandum of Understanding between the US Army Environmental Hygiene Agency; the US Army Health Services Command; the Department of the Army; Office of The Surgeon General; the Armed Forces Pest Control Board; and the US Department of Agriculture, Agricultural Research, Science and Education Administrations; titled, Coordination of Biological and Toxicological Testing of Pesticides, effective 23 January 1979.

b. Letter, AFPCB, Armed Forces Pest Control Board, 17 March 1977, subject: Reregistration Data for N,N-Diethyltoluamide Reppellent.

2. REFERENCES.

a. Report, USAEHA-LT, this Agency, 11 October 1974, Study No. 51-051-73/75, Development of an Efficient Test System for Assessing Behavioral Effects of Exposure to Chemical Compounds, November 1972 - November 1973.

b. Report, HSE-LT/WP, this Agency, 8 May 1979, Preliminary Behavioral Assessment of Habituation to the Insecticide Permethrin, Study No. 75-51-0026-79, August - October 1978.

c. Report, HSE-LT/WP, this Agency, 14 November 1979, Toxicological Assessment Program Study No. 75-51-0034-80, Behavioral Effects of Acute Aerosol Exposure to N,N-Diethyl-Meta-Toluamide (M-Det), January - February 1979.

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\* The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," US Department of Health, Education and Welfare, Publication No. (NIH) 78-23, revised 1978.

† This study was performed in animal facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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d. Report, HSE-LT/WP, this Agency, Phase 4, Inhalation Toxicities of N,N-Diethyl-Meta-Toluamide (M-Det), Study No. 75-51-0034-80, January - May 1979, in preparation.

3. PURPOSE. To determine whether a battery of behavioral tests could detect changes in rats exposed for 13 weeks to aerosols of M-Det (an insect repellent). The tests provided support for the standard physiological, chemical, and observational measures carried out concurrently with the same rats.

4. BACKGROUND. The Toxicology Division, US Army Environmental Hygiene Agency (USAEHA), is evaluating the effects of subchronic aerosol exposure to M-Det (paragraph 2d). With the exception of our acute study, previous studies of M-Det have not addressed themselves to its behavioral effects (paragraph 2c). As behavioral effects were found in our acute exposure study, behavioral measures appeared to be warranted in the subchronic study and were carried out concurrently with the standard chemical, physical, and observational tests using the same rats. The results of the nonbehavioral tests are reported separately (paragraph 2d).

5. PROCEDURE.

a. Relationship to the Full Study. The procedures described here are only those applicable to the behavioral parts of the study. The full subchronic study included other animals and techniques which did not affect its behavioral aspects. These procedures as well as animal marking and care, the test material, details of the chambers, and sampling methodology are described in the report of the entire study (paragraph 2d).

b. Exposure Groups and Procedures. Four groups of 10 males and 10 female rats were exposed for 13 weeks to three aerosol concentrations of M-Det plus an air-only chamber control. All rats were kept in similar chambers for 6 hours per day, 5 days per week. M-Det concentrations averaged about 1500 mg/m<sup>3</sup> (1/4 LC50), 750 mg/m<sup>3</sup> (1/8 LC50), and 250 mg/m<sup>3</sup> (1/24 LC50).

c. Toxic Signs. The personnel carrying out behavioral tests looked for 16 commonly used toxic signs and other abnormalities in each rat when they were removed from the exposure chambers on days behavioral testing was to be done. These signs are described in Table 1. Toxic signs were also looked for among groups after every day's exposure by the personnel running the chambers.



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TABLE 1. TOXIC SIGNS

1. Abnormal salivation.
2. Frequent large swallowing movements and coughing.
3. Lacrimation and eye bulb protrusion.
4. Abnormally frequent urination.
5. Fasciculation (twitching) - (a) local and (b) generalized.
6. Tremors (shivering) - (a) head only and (b) whole body.
7. Eye blink reflex.
8. Hyperreactivity (exaggerated startle).
9. Prostration.
10. Seizures.
11. Loss of balance while walking.
12. Frequent or almost continuous conflict during active periods.
13. Radical changes in the time of active periods.
14. Obvious changes in the amount of overall activity.
15. Grip strength as measured by resistance to pull from a grid.
16. Eyes partially or entirely closed.

d. Behavioral Measures. The six tests which proved sensitive to M-Det's effects during the acute study (paragraph 2c) were used in this study. Those tests, the results of which were not likely to be significantly affected by biweekly repetition, were presented on weeks 2, 4, 6, 8, 11, and 13 of the study, while those tests likely to be affected were only presented during week 13. Because time between the end of exposure and the start of any test may have been critical (i.e., results were likely to vary with time), all rats in each exposure group were run through each test at the same time. Thus, all rats in a group received any given test within a maximum of 15 minutes of each other, and all groups received that particular test at about the same amount of time after the end of the exposure session. The behavioral tests are described in great detail in the acute M-Det report (paragraph 2c). The schedule of test presentation is illustrated in Figure 1.

(1) Behavioral Measures Presented on Alternate Weeks (Including Week 13).

- (a) Endurance - grip strength.
- (b) Tactile sensitivity (pain sensitivity and reactions).
- (c) Balance beam (vestibular system and coordination).

(2) Behavioral Measures Presented Only During the 13th Week of the Study.

- (a) Quick avoidance (memory).



A. GROUPS:

| GROUP   | M-DET CONCENTRATION<br>MG/M <sup>3</sup> | FRACTION OF LC50 | #<br>MALES | #<br>RATS<br>FEMALES |
|---------|--|------------------|------------|----------------------|
| control | 0  | 0                | 10         | 10                   |
| low     | 250                                      | 1/24             | 10         | 10                   |
| medium  | 750                                      | 1/8              | 10         | 10                   |
| high    | 1,500                                    | 1/4              | 10         | 10                   |

B. EXPOSURE AND BEHAVIORAL TEST SCHEDULE

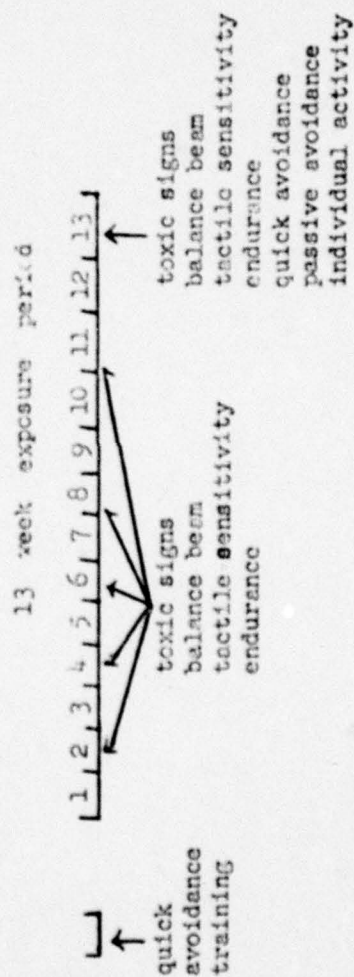


Figure 1. Experimental Design

(b) Passive avoidance (post exposure learning).

(c) Individual short-term activity (tremors, locomotion).

6. STATISTICS. Throughout the study, statistical significances among groups are beyond the 0.01 probability level whenever groups are said to be different from each other. Differences among groups were tested with analysis of variance and student's "t" tests when data were normally distributed and groups had similar variance; when variance differed, nonparametric tests were used. Pearson's correlations were used to establish concentration response relationships. See paragraph 2c for a discussion of our statistical methods and reasoning. When group means are presented, they are always followed by their standard deviations in parenthesis.

## 7. RESULTS.

a. Nonbehavioral Measures. There were no obvious abnormalities among the necropsied rats, and no rats died during the study. The toxic signs looked for by the personnel carrying out behavioral tests did not show differences beyond the fur of higher concentration groups being disheveled and discolored after exposure. At the end of the study, there were no significant differences in weight among any groups of the same sex.

b. Behavioral Measures Overview. When the results of the entire battery are considered, the effects of all the concentrations could definitely be differentiated from each other, but no one test definitely differentiated among all four levels. The differential ability of the tests and a summary of test results appear in Table 2. The rats showed a definite change over time with some early differences disappearing, while others only appeared towards the end of the study.

### c. Behavioral Tests Given Only at the End of the Study.

(1) Passive Avoidance. Among males, five controls (half) failed to learn to avoid the shock while no low-concentration, one medium-concentration, and two high-concentration group rats failed to learn. None of the other variables measured were significantly different. These data are summarized in Table 3 and illustrated in Figure 2.

(2) Quick Avoidance. During preexposure training, there were no differences among any group of one sex. On the third postexposure test trial, male controls were significantly slower at leaving the start box than the low-concentration group which were, in turn, slower than the medium- and high-concentration groups ( $t = 3.83$ , 18 DF,  $p 0.01$ ). The number of rats shocked by remaining in the start box for more than 20 seconds paralleled the time required to leave. No other variables were significantly different. The data are summarized in Table 4 and illustrated in Figure 3.

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TABLE 2. TEST AND CONCENTRATION DIFFERENTIATION SUMMARY

Lines between concentration levels indicate significant differences detected by a test. A lack of lines indicates no difference. C = control (no M-Det), L = low, M = medium, and H = high concentration. For example, c-lmh means that the control group is different from the low, medium and high groups which are statistically similar to each other. Concentrations in the acute study were far higher than those in the subchronic study.

| <u>TEST</u>            | <u>SEX</u> | <u>ACUTE</u>        | <u>SUBCHRONIC</u> |
|------------------------|------------|---------------------|-------------------|
| Endurance              | F          | c-l-m-h             | c-l-m-h           |
|                        | M          | cm-l, cm-h, l-h, mh | clmh              |
| Passive<br>avoidance   | F          | c-lm-h              | c-lmh             |
|                        | M          | clmh                | c-lmh             |
| Quick<br>avoidance     | F          | clm-h               | c-lmh             |
|                        | M          | cl-mh               | c-l-mh            |
| Balance                | F          | c-lm-h              | c-l-m-h           |
|                        | M          | c-lm-h              | clmh              |
| Activity               | F          | clm-h               | clmh              |
|                        | M          | clmh                | clmh              |
| Tactile<br>sensitivity | F          | clmh                | clmh              |
|                        | M          | c-lmh               | clmh              |



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TABLE 2. TEST AND CONCENTRATION DIFFERENTIATION SUMMARY

Lines between concentration levels indicate significant differences detected by a test. A lack of lines indicates no difference. C = control (no M-Det), L = low, M = medium, and H = high concentration. For example, c-lmh means that the control group is different from the low, medium and high groups which are statistically similar to each other. Concentrations in the acute study were far higher than those in the subchronic study.

| <u>TEST</u>         | <u>SEX</u> | <u>ACUTE</u>        | <u>SUBCHRONIC</u> |
|---------------------|------------|---------------------|-------------------|
| Endurance           | F          | c-l-m-h             | cl-m-h            |
|                     | M          | cm-l, cm-h, l-h, mh | clmh              |
| Passive avoidance   | F          | c-lm-h              | c-lmh             |
|                     | M          | clmh                | c-lmh             |
| Quick avoidance     | F          | clm-h               | c-lmh             |
|                     | M          | cl-mh               | c-l-mh            |
| Balance             | F          | c-lm-h              | cl-m-h            |
|                     | M          | c-lm-h              | clmh              |
| Activity            | F          | clm-h               | clmh              |
|                     | M          | clmh                | clmh              |
| Tactile sensitivity | F          | clmh                | clmh              |
|                     | M          | c-lmh               | clmh              |



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TABLE 3. PASSIVE AVOIDANCE\*

| SEX | GROUP   | # SHOCKS | # SECONDS<br>OUT OF<br>SAFE AREA | # SECONDS<br>BET LST<br>SHOCK & END<br>OF SESSION | # TIMES LEFT<br>SAFE AREA | # DID NOT<br>LEARN† |
|-----|---------|----------|----------------------------------|---|---------------------------|---------------------|
| M   | Control | 6(5)     | 38(31)                           | 29(36)  | 9(6)                      | 5                   |
| M   | Low     | 5(3)     | 24(19)                           | 61(27)  | 7(5)                      | 0                   |
| M   | Medium  | 5(3)     | 27(13)                           | 51(32)  | 8(5)                      | 1                   |
| M   | High    | 8(5)     | 41(26)                           | 32(22)  | 10(4)                     | 2                   |
| F   | Control | 10(4)    | 48(22)                           | 17(22)  | 13(5)                     | 6                   |
| F   | Low     | 9(4)     | 57(30)                           | 35(33)  | 9(5)                      | 3                   |
| F   | Medium  | 8(4)     | 44(27)                           | 29(24)  | 10(4)                     | 3                   |
| F   | High    | 9(4)     | 46(20)                           | 38(25)  | 10(5)                     | 2                   |

\* Group means are presented with their standard deviations in brackets.

† Number of rats in a group of 10 which were out of the safe area for at least 5 seconds at the end of the session.

TABLE 4. QUICK AVOIDANCE

| SEX | GROUP<br>(10 Rats) | SECONDS TO LEAVE START BOX IN TRIAL #: |      |        | # SHOCKED |
|-----|--------------------|--|------|--------|-----------|
|     |                    | 1                                      | 2    | 3      |           |
| M   | Control            | 2(2)                                   | 4(5) | 16(11) | 4         |
| M   | Low                | 5(9)                                   | 3(5) | 6(9)   | 2         |
| M   | Medium             | 5(9)                                   | 2(1) | 2(2)   | 1         |
| M   | High               | 3(2)                                   | 3(3) | 2(2)   | 0         |
| F   | Control            | 4(4)                                   | 3(5) | 4(6)   | 2         |
| F   | Low                | 3(4)                                   | 1(1) | 2(3)   | 0         |
| F   | Medium             | 2(2)                                   | 3(4) | 5(7)   | 0         |
| F   | High               | 2(3)                                   | 2(5) | 2(3)   | 0         |

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NUMBER OF RATS IN EACH GROUP WHO DID NOT LEARN

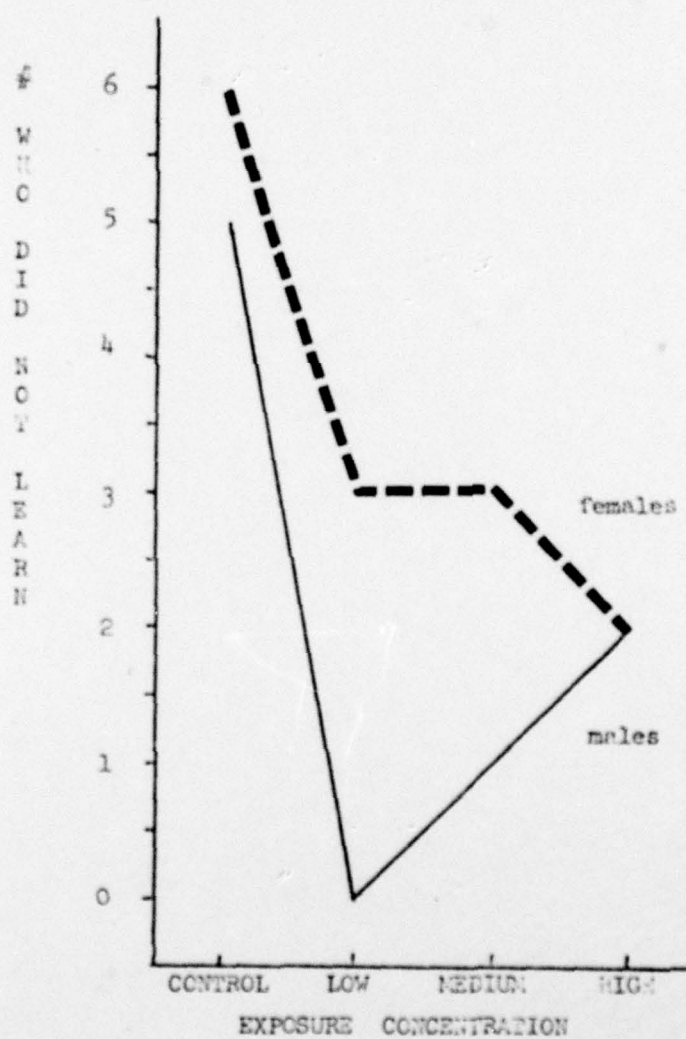


Figure 2. Passive Avoidance

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Male's trial three. Bars indicate average number of seconds to leave the start box. Lines are the group standard deviations. Numbers near the bars' tops indicate the number of rats in each group remaining in the start box more than 20 seconds.

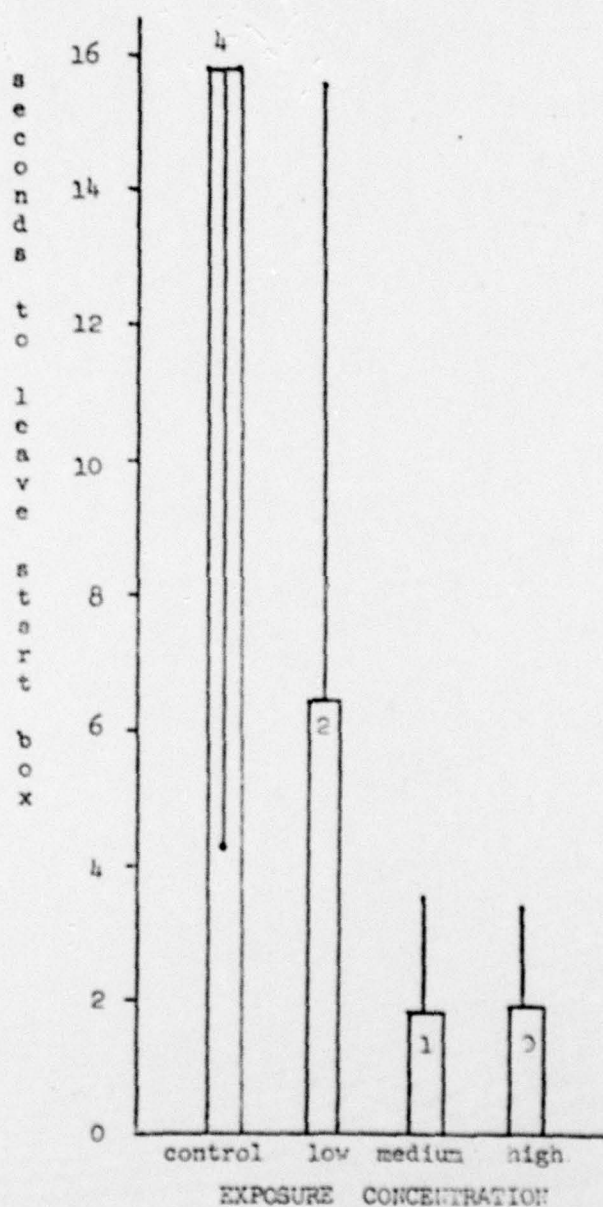


Figure 3. Quick Avoidance



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(3) Individual Short-term Activity. There was no significant differences among any of the groups at any level of activity. These data are summarized in Table 5.

d. Behavioral Tests Given Throughout the Study.

(1) Endurance. Among females, during the second week of exposure, all groups were statistically similar but, by the fourth week, a clear response was evidenced which was maintained to varying extents throughout the rest of the study. The trend was unusual in that the low-concentration group hung onto the rod longer than the controls in five of the six observations. The difference was significant on the 11th week (52 vs. 41 seconds). The control and low-concentration groups showed a significant rising trend in ability to hold the bar which stabilized at 6 weeks. The medium-concentration group showed an increase from week 2 to week 4, but then dropped off as the study progressed. The high group was stably low (about 24 seconds) from weeks 2 through 6, and then dropped significantly to a lower level of about 14 seconds which remained stable from week 8 to week 13. The males did not show a parallel response. On the first test (week 2), the high group held on significantly longer than the controls with the medium and low groups falling together in between. By the sixth and eighth weeks, this trend was reversed, with the controls holding on longer than any other groups. The trend changed again so that by week 13, the low dose held on longer than others, who were similarly low. These trends were paralleled by trends in the number of rats holding on for more than 30 seconds. These data are summarized in Table 6 and illustrated in Figures 4 and 5.

(2) Balance. Among females, all groups were similar at the second week. By the fourth week, fewer members of the medium group moved beyond the beam's start point than the other. By the eighth week, a clear trend differentiating the medium (six not moving) from the control (two not moving) and low (one not moving) from the high (none moving) group was established and maintained for the rest of the study. All of the female members of the high group moved on each test while the low and control groups always had some not moving, so the difference among them is numerically small but consistent. The low and control groups could not be distinguished from each other throughout the study by balance measures. The male groups showed a processing number of rats not moving, with the medium group moving least by the eighth week. The trends in the number of rats which walked off were the opposite of those for the number not moving, except the high group could not be differentiated from the low and control groups even in the later weeks of the study. Medium group females remained at about four walking off through the study, while the others had increased to about eight by the end of the fourth week. The difference was maintained until the 13th week when the



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TABLE 5. EXPLORATORY ACTIVITY

| SEX | GROUP<br>(OF 10) | # VIBRATIONS | # MEDIUM<br>MOVEMENTS | # LARGE<br>MOVEMENTS | # URINATED | # BOLT |
|-----|------------------|--------------|-----------------------|----------------------|------------|--------|
| M   | Control          | 415(115)     | 73(38)                | 54(29)               | 3          | 1      |
| M   | Low              | 383(130)     | 64(39)                | 37(22)               | 3          | 10     |
| M   | Medium           | 429(123)     | 50(27)                | 31(15)               | 2          | 9      |
| M   | High             | 434(101)     | 87(37)                | 49(18)               | 2          | 1      |
| F   | Control          | 405(140)     | 79(34)                | 45(19)               | 1          | 0      |
| F   | Low              | 436(109)     | 74(34)                | 43(26)               | 6          | 0      |
| F   | Medium           | 399(106)     | 71(38)                | 37(18)               | 0          | 0      |
| F   | High             | 389(77)      | 69(21)                | 36(12)               | 2          | 0      |

TABLE 6. GRIP STRENGTH/ENDURANCE - Average No. Seconds (+ Standard Deviation) Suspended from Horizontal Bar

| SEX | GROUP   | TEST WEEK |         |         |         |         |         |
|-----|---------|-----------|---------|---------|---------|---------|---------|
|     |         | 2         | 4       | 6       | 8       | 11      | 13      |
| M   | Control | 9 (3)     | 17 (12) | 23 (17) | 27 (17) | 22 (18) | 13 (17) |
| M   | Low     | 14 (9)    | 20 (16) | 11 (4)  | 13 (8)  | 20 (16) | 21 (18) |
| M   | Medium  | 15 (10)   | 12 (10) | 10 (7)  | 13 (11) | 15 (13) | 14 (10) |
| M   | High    | 20 (18)   | 17 (22) | 12 (13) | 10 (8)  | 12 (9)  | 16 (17) |
| F   | Control | 15 (15)   | 36 (20) | 47 (16) | 41 (20) | 41 (20) | 38 (21) |
| F   | Low     | 19 (20)   | 41 (20) | 53 (9)  | 32 (22) | 52 (15) | 40 (23) |
| F   | Medium  | 19 (18)   | 33 (24) | 35 (21) | 28 (21) | 24 (20) | 25 (24) |
| F   | High    | 25 (21)   | 25 (20) | 23 (20) | 15 (12) | 16 (17) | 14 (17) |

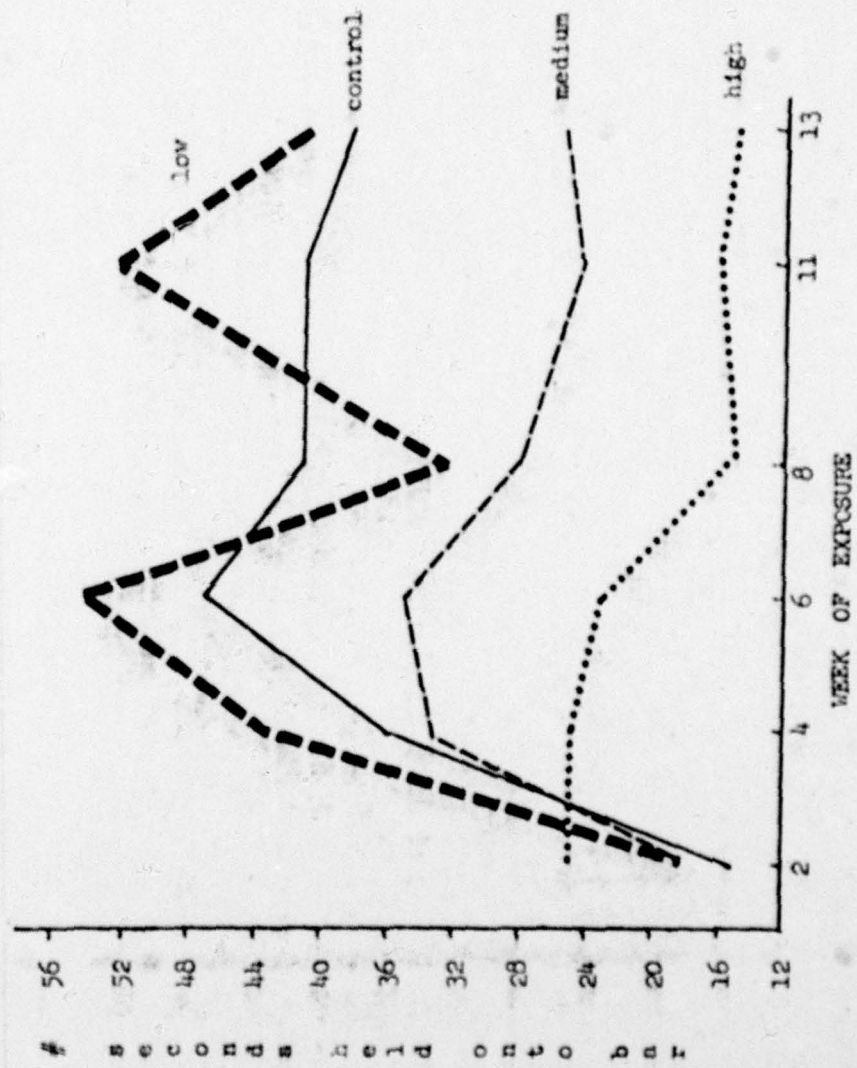


FIGURE 4. Endurance - number of seconds females were able to hang onto the bar (group average).

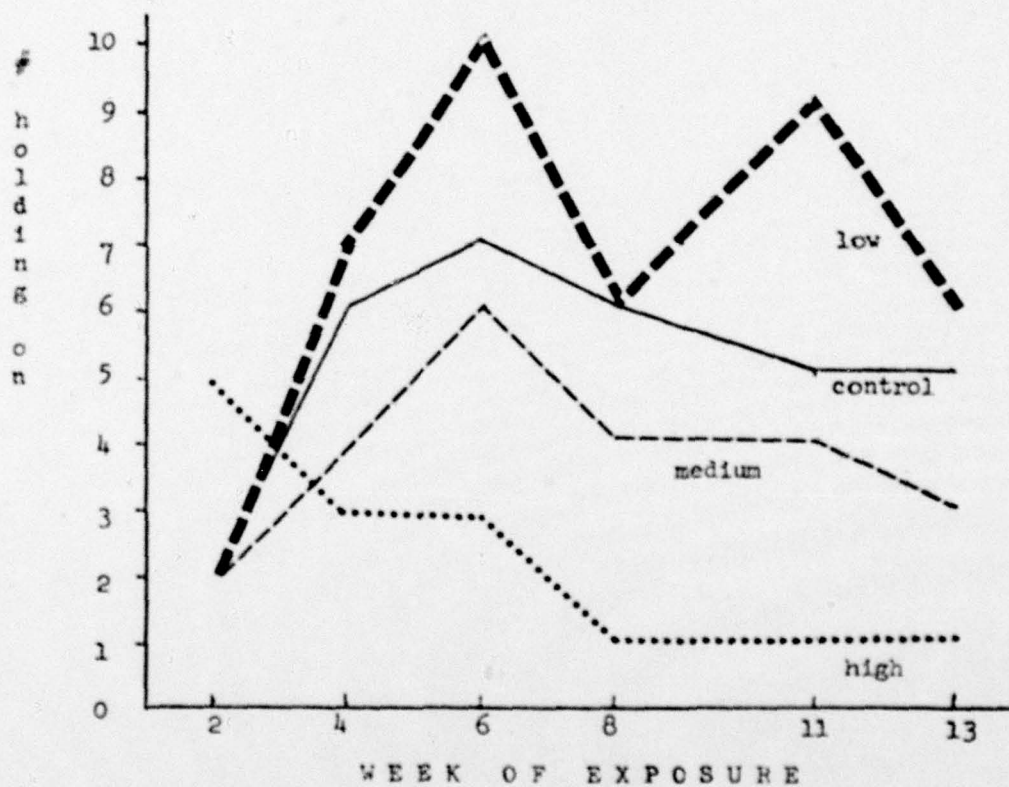


FIGURE 5. Endurance - number of females in each group holding onto bar for more than 30 seconds.



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control, low, and high groups showed decreases of two, one, and three animals, respectively, walking off. These data are summarized in Table 7 and illustrated in Figures 6 and 7.

(3) Tactile Sensitivity. Although all groups of both sexes showed significant decreasing trends in time to respond to heat from beginning to end of the study, there were no differences among the groups. The data are summarized in Table 8.

#### 8. DISCUSSION.

a. Effects of Changes in Tactile Sensitivity on Other Behavioral Measures. The significant trend on the part of all groups to respond more quickly to heat as the study progressed probably does not indicate an increase in tactile sensitivity but, rather, that all groups learned that the faster they responded, the faster they got away from the discomfort. A difference in tactile sensitivity in which controls were the least sensitive would have explained the control groups not learning the postexposure passive avoidance task as well as the other groups, because they would not have been as affected by the shock. However, this was not the case. The high group's fur may have been wetter than the others as they were more ruffled and damp-looking than the others. If they were wetter, their skin and fur would have conducted electricity better so they would have been more sensitive to the shock and more stimulated to learn. However, the high male group did not learn as well as the low and medium groups. Perhaps two variables (shock sensitivity and effect of M-Det) balanced out. Difference in shock sensitivity due to wetness or other factors would not have caused the controls to require more time to leave the start box during the quick avoidance trials because this was a memory task with no shock involved for the first 20 seconds. Virtually all the rats exposed to M-Det were out of the box before the shock started.

b. Relationship Between Size and Weight of Rat and Study Results. During the course of the study, the rats gained considerable weight and size. The males went from an average of about 121 gm (SD = 9) to an average of 471 gm (SD = 22), while the females changed from 113 gm (SD = 11) to 291 gm (SD = 17). This difference should affect some test results. For example, strength usually does not increase proportionately with weight so, as weight increases, endurance rod performance should decrease or not increase drastically. In fact, this trend did not appear. An increase in size should have made the balance beam more difficult to walk across as it became relatively narrower as size increased. However, weight was not related to walking off the beam. Thus, the increase in size and weight during the study did not appear to affect the data in any obvious way.

TABLE 7. BALANCE BEAM

| SEX | GROUP   | TEST WEEK  |                    |            |                    |            |                    |            |                    |            |                    |            |                    |   |   |   |   |    |   |   |
|-----|---------|------------|--------------------|------------|--------------------|------------|--------------------|------------|--------------------|------------|--------------------|------------|--------------------|---|---|---|---|----|---|---|
|     |         | 2          |                    | 4          |                    | 6          |                    | 8          |                    | 11         |                    | 13         |                    |   |   |   |   |    |   |   |
|     |         | LST<br>BAL | DNM<br>WLKD<br>OFF | LST<br>BAL | DNM<br>WLKD<br>OFF | LST<br>BAL | DNM<br>WLKD<br>OFF | LST<br>BAL | DNM<br>WLKD<br>OFF | LST<br>BAL | DNM<br>WLKD<br>OFF | LST<br>BAL | DNM<br>WLKD<br>OFF |   |   |   |   |    |   |   |
| M   | Control | 7          | 3                  | 3          | 3                  | 2          | 7                  | 1          | 2                  | 8          | 1                  | 0          | 7                  | 2 | 2 | 8 | 1 | 6  | 1 |   |
| M   | Low     | 8          | 0                  | 4          | 4                  | 4          | 3                  | 4          | 3                  | 6          | 3                  | 1          | 5                  | 2 | 0 | 6 | 1 | 6  | 1 |   |
| M   | Medium  | 9          | 2                  | 1          | 3                  | 3          | 3                  | 0          | 0                  | 6          | 0                  | 1          | 10                 | 0 | 0 | 9 | 0 | 10 | 0 |   |
| M   | High    | 6          | 1                  | 3          | 3                  | 2          | 3                  | 3          | 3                  | 2          | 2                  | 4          | 4                  | 1 | 1 | 4 | 2 | 8  | 1 |   |
| F   | Control | 2          | 2                  | 5          | 0                  | 1          | 1                  | 9          | 0                  | 0          | 0                  | 0          | 2                  | 8 | 4 | 2 | 8 | 1  | 2 | 6 |
| F   | Low     | 7          | 2                  | 3          | 0                  | 0          | 0                  | 7          | 2                  | 1          | 9                  | 0          | 1                  | 8 | 0 | 2 | 2 | 2  | 7 | 7 |
| F   | Medium  | 6          | 1                  | 4          | 4                  | 4          | 4                  | 3          | 2                  | 2          | 4                  | 0          | 6                  | 4 | 1 | 5 | 4 | 6  | 4 | 4 |
| F   | High    | 2          | 0                  | 6          | 0                  | 0          | 0                  | 9          | 3                  | 0          | 7                  | 1          | 0                  | 8 | 4 | 0 | 2 | 0  | 5 | 5 |

LST BAL - Number of times rats in a group of 10 lost their balance.  
DNM - Number of rats in a group who did not move past the start point.  
WLKD OFF - Number of rats in a group who walked off the beam.  
One medium concentration male fell off during weeks two and four.

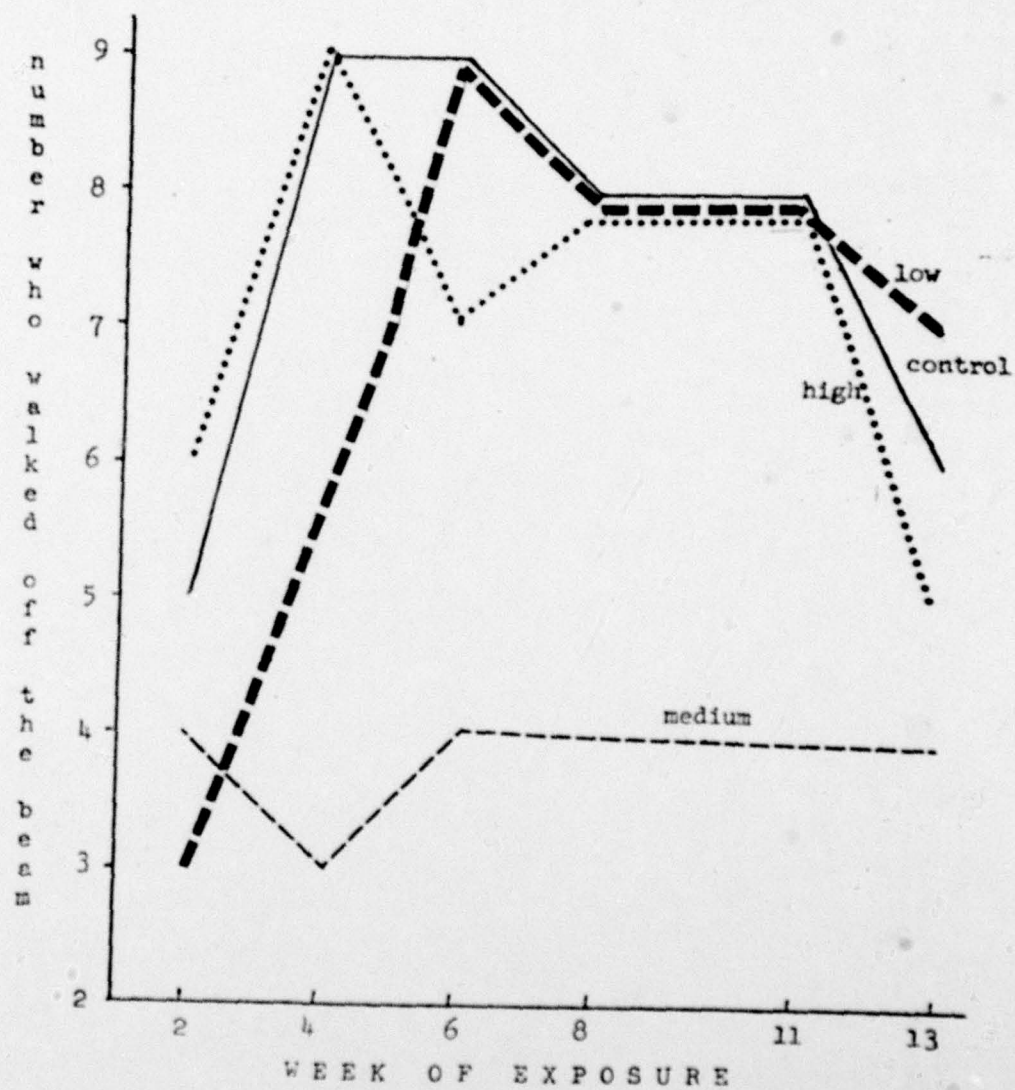


FIGURE 6. Balance - number of females who walked off the beam.



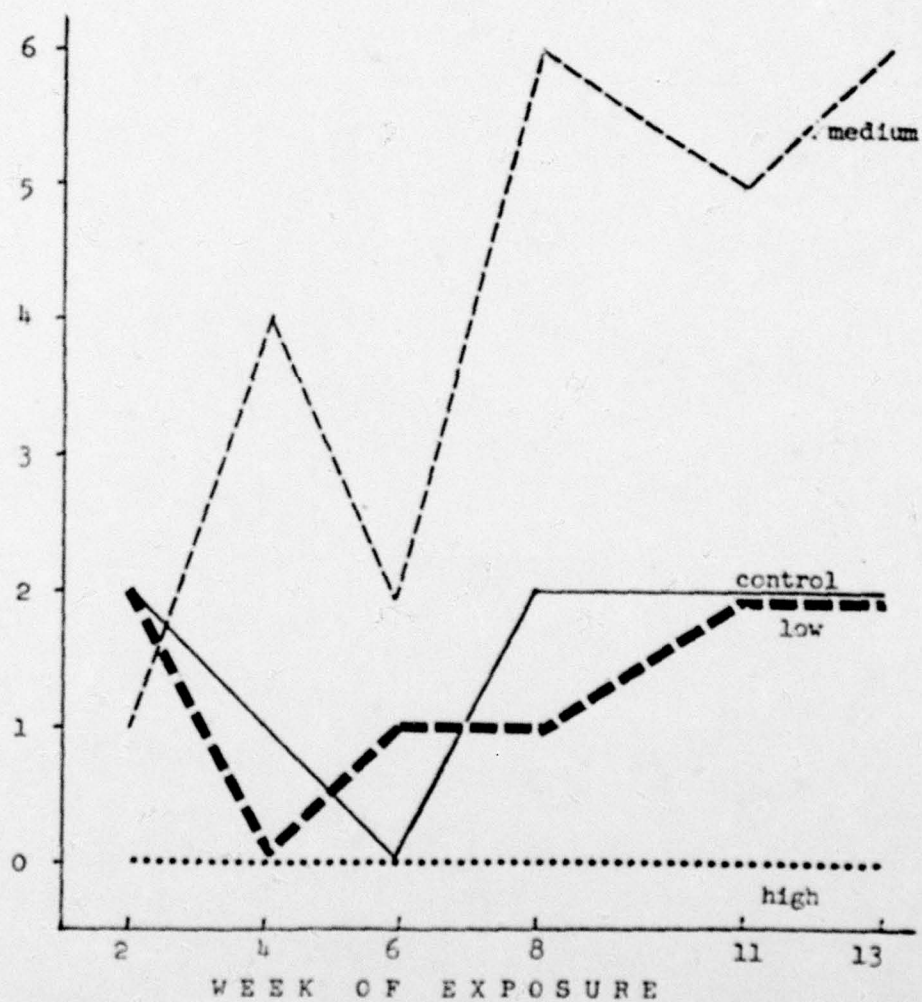


FIGURE 7. Balance - number of females who did not move from the beam's start point.

8. TACTILE SENSITIVITY

| SEX | EXPOSURE<br>CONCENTRATION | EXPOSURE WEEK |           |          |           |          |          |          |          |          |          |          |          |
|-----|---------------------------|---------------|-----------|----------|-----------|----------|----------|----------|----------|----------|----------|----------|----------|
|     |                           | 1             |           |          | 2         |          |          | 3        |          |          | 4        |          |          |
|     |                           | S             | L         | II       | S         | L        | II       | S        | L        | II       | S        | L        | II       |
| M   | Control                   | 7<br>(3)      | 9<br>(4)  | 8<br>(4) | 8<br>(4)  | 3<br>(2) | 5<br>(2) | 4<br>(3) | 1<br>(3) | 3<br>(2) | 4<br>(3) | 2<br>(4) | 7<br>(9) |
| M   | Low                       | 6<br>(3)      | 8<br>(3)  | -        | 8<br>(3)  | 6<br>(5) | 6<br>(5) | 5<br>(2) | 5<br>(2) | 5<br>(3) | 4<br>(2) | 4<br>(2) | 4<br>(5) |
| M   | Medium                    | 7<br>(2)      | 9<br>(3)  | -        | 8<br>(3)  | 3<br>(3) | 6<br>(3) | 4<br>(2) | 2<br>(2) | 3<br>(3) | 4<br>(3) | 1<br>(2) | 3<br>(1) |
| M   | High                      | 7<br>(2)      | 6<br>(2)  | -        | 7<br>(4)  | 1<br>(2) | 4<br>(2) | 3<br>(3) | 1<br>(3) | 2<br>(2) | 3<br>(4) | 1<br>(5) | 3<br>(2) |
| F   | Control                   | 7<br>(4)      | 10<br>(3) | -        | 9<br>(5)  | 7<br>(3) | 6<br>(3) | 5<br>(2) | 4<br>(2) | 4<br>(2) | 5<br>(3) | 3<br>(3) | 3<br>(2) |
| F   | Low                       | 7<br>(2)      | 6<br>(2)  | -        | 9<br>(4)  | 6<br>(2) | 4<br>(2) | 4<br>(2) | 2<br>(2) | 4<br>(4) | 4<br>(3) | 2<br>(4) | 4<br>(1) |
| F   | Medium                    | 8<br>(4)      | 10<br>(3) | -        | 11<br>(6) | 6<br>(2) | 7<br>(2) | 5<br>(3) | 4<br>(2) | 3<br>(4) | 5<br>(2) | 3<br>(3) | 2<br>(2) |
| F   | High                      | 9<br>(4)      | 8<br>(3)  | -        | 14<br>(8) | 5<br>(3) | 6<br>(3) | 4<br>(3) | 1<br>(3) | 2<br>(1) | 3<br>(2) | 0<br>(2) | 1<br>(1) |

S = Average number of seconds to respond  $\pm$  standard deviation.

L = Number of rats in group of 10 who responded by licking a hind paw. The others responded by jumping out of the test box.

c. Concentration Response Order Anomalies. During many repetitions of the endurance and balance test, the M-Det-exposed and control groups could be differentiated from each other, but their level of response was not in any predictable order. In the balance test, the medium group was out of place, while the low group was out of place in the endurance test. These results suggest that M-Det does not simply increase or decrease responses in proportion to exposure level but, rather, may have an activating effect at low levels and a deactivating effect at higher levels. Thus, typical dose-response relationships, in which regression can be used to predict responses at doses other than those tested, were not demonstrated for these levels of M-Det exposure. However, many of the test results from the acute study (paragraph 2c) showed typical dose-response relationships at far higher concentrations.

d. Relationship of the Acute Study to the Subchronic Study. The acute study (paragraph 2c) found differences among exposure levels for every behavior tested in this subchronic study. However, the exposure concentrations were much higher with the range being 4,100 to 2,300 mg/m<sup>3</sup>, while the subchronic levels ranged between 1,500 and 250 mg/m<sup>3</sup>. Thus, it is not surprising that the results of the subchronic study are less dramatic than those of the acute study. The initial measures taken 2 weeks into the subchronic study cannot, of course, be used as equivalents of low acute exposure for direct comparison with the higher acute exposure levels due to the possibility of habituation to the chemical as occurred with Permethrin (paragraph 2c) and/or cumulative effects. That effects are still present at these low levels is still important. The results of the two studies are contrasted in Figure 2.

e. Relationship of Experimental Exposure Levels to Likely Environmental Exposure Levels. A typical 56-gram aerosol can (e.g., National Stock Number 6840-00-864-5434) contains about 40 grams of material at a M-Det concentration of 71 percent. A typical spray nozzle emits about 1 gram per second. It takes about 10-seconds to spray oneself with an insecticide. If this was done in a small 10 m<sup>3</sup> room, the occupant would be exposed to a concentration of about 0.7 grams per cubic meter of M-Det. The 250 mg/m<sup>3</sup> level is 2.8 times less than the concentration one would be exposed to when using the spray in normal use; but, exposure was for hours as opposed to seconds. However, if M-Det produces its major effect through skin penetration rather than inhalation, the expected use levels could be similar to the medium concentration depending on how much is actually applied and how often applications are repeated. One container of repellent (NSN 6840-00-753-4963) calls for the use of about 2 ounces per day per person for some applications. At 71.25 percent M-Det concentration, this is 40 grams. The container called for using 12 drops, which weighed about 0.4 grams, to be used per application. This is actually far less than is likely to be squirted into the hand when starting to apply M-Det from this type of squeeze bottle. The frequency of repetition is not specified.



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f. Value of a Multitest Screen. The key to the value of a multiple test screen is the ability to reliably differentiate between many doses of a compound using tests which are individually able to show consistent differences between only several of the many doses. The compilation of results from individual consistent tests gives consistent results over a far wider range than an individual test could have provided. A single rather ineffective test repeated many times might randomly show some groups as being apparently different than others in random directions. No one test used in this study differentiated between all groups of both sexes all the time. However, individual tests did produce consistent, statistically significant differences between several groups over a period of time. When the results of all the tests were compiled, all variables were readily differentiable from each other. Thus, the true value of the screen is that it can do more than any one test and do it consistently, quickly, and effectively. This compilation is presented in Table 8.

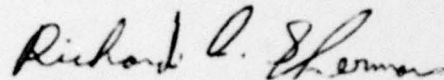
g. Possible Relationships Between Changes in Rats' Performance on Behavioral Tests After Exposure to M-Det and Human Health and Function. M-Det has been in use for many years and no reports have appeared in the literature indicating behavioral effects in either humans or other mammals. This would indicate little potential for causing major, readily observable problems at current use concentrations under normal circumstances. However, these high concentration acute and subchronic tests at very high exposure concentrations have demonstrated effects clearly deleterious to performance under conditions when optimal function would be vital to success. For example, endurance and balance are effected and even unnoticeable changes in these variables may produce decrements in performance of tasks requiring a high degree of sustained coordination. These animal tests do not demonstrate a direct effect on human health or function but may indicate potential for diminution of critical functioning following prolonged or frequently repeated use of this repellent.

9. CONCLUSION. Behavioral changes due to aerosol exposure to M-Det over a period of 13 weeks were demonstrated. When the results of the entire battery were used, all levels could be differentiated from each other and from air controls. Among some measures, these differences changed during the course of exposure.

10. RECOMMENDATIONS. Because the changes were significant in both the acute and subchronic study, but not in a clear dose-response type of relationship, it is recommended that this compound be studied further by other laboratories with the facilities to carry out an indepth analysis of its behavioral effects. The lack of a true dose-response relationship among the results precludes extrapolation of these results to possible lower concentration effects. No change in use of products containing M-Det is recommended at

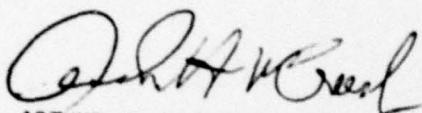
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this time because the only concentration low enough to be within the range of normal use (250 mg/m<sup>3</sup>) was not significantly different from the controls for most tests until 6 weeks of exposure had been completed.



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